The role of nitrogen fixation in primary succession on land

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SUMMARY

1 The possible role of nitrogen-fixing organisms in primary succession is evaluated, principally for sites on sandy shores (intertidal and above high tide) and volcanic lava.

2 Cyanobacteria are common on most sites. How much nitrogen they fix and how much of this is made available to subsequent stages in succession is unclear. At its maximum level, cyanobacterial fixation could support growth of some angiosperms.

3 Legume and non-legume nodulated plants are comparatively rare in the early stages of primary succession. They may, however, establish successfully when seed is introduced. The presence of an endophyte rarely appears to be a limiting factor in establishment.

4 The possible consequences of nodulated plant colonization during early succession are briefly discussed.

INTRODUCTION

Nitrogenase, the enzyme that converts N_2 gas into ammonium, is big, slow and expensive to operate (Sprent & Raven 1985). It follows that nitrogen-fixing organisms will only have a significant role to play when nitrogen is the major factor limiting colonization and there is a plentiful supply of energy (either direct, e.g. light, or indirect, e.g. reduced carbon). These conditions do not always occur in the early stages of succession and even when they do, nitrogen-fixing organisms do not necessarily occur.

This chapter considers where, when, why and which nitrogen-fixing organisms are involved in primary succession. It will do so by discussing a selection of examples covering a range of nitrogen-fixing organisms and some major natural pioneer habitats. For more detail of the biology of the organisms involved, see Sprent & Sprent (1990). An exhaustive survey of the occurrence of nitrogen-fixing organisms in different primary successions is given by Walker (1993).

SANDY SHORES

By their very nature, sandy shores have an interface with the intertidal zones.

Recent work has analysed the laminated microbial mats which occur in temperate and tropical beaches. They consist of a series of layers, the exact order of which may vary according to physicochemical properties (oxygen and light penetration, etc.) but which usually have layers of green algae/cyanobacteria, purple sulphur bacteria and sulphate-reducing bacteria. Occasionally the green algal/cyanobacterial component is absent (Herbert *et al.* 1988). Fig. 1 illustrates the main features of a classical microbial mat, found in many sheltered beaches, such as the island of Mellum in the bay of Bremerhaven in the North Sea (Stal 1985). These, theoretically at least, are self-perpetuating.

The upper layer contains cyanobacteria, the exact species composition of which varies: these fix both carbon dioxide and nitrogen. The lower cells die, releasing organic carbon, which is metabolized by aerobic organisms, eventually generating anaerobic conditions. Sulphate, which is plentiful, can then be used by sulphate-reducing species, generating sulphide, which in turn forms a substrate for the anoxic photosynthetic purple sulphur bacteria. In an accreting system the organic nitrogen of such mats could form a rich substrate for land-colonizing plants. Unlike many other situations involving nitrogen-fixing cyanobacteria, no heterocystous forms were found by Stal (1985) at Mellum, possibly because of the high levels of sulphide (Malin & Pearson 1988).

SAND ABOVE THE TIDEMARK

In terms of primary colonization, coastal sand is arguably most important. However, current concern over 'desertification' and its reversal has enhanced consideration of nitrogen fixation in inland sandy areas (which may or may not be saline as well as arid). Consideration of both inland and coastal sandy areas allows some separation of factors, which may influence the occurrence and type of nitrogen-

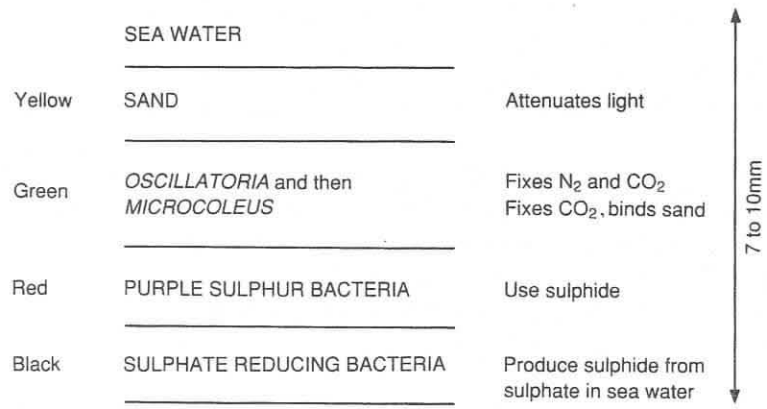

FIG. 1. Some features of the microbial mat on Mellum island (Stal 1985). For variations see Herbert *et al.* (1988).

fixing organisms found. The major factors are texture, water, mineral content (including nitrogen), salinity, mobility and their interactions.

The effects of soil texture and cation exchange capacity on the occurrence of the nitrogen-fixing cyanobacterium *Anabaena variabilis* were considered by Skujins *et al.* (1987). Texture was varied by combining acid-washed and neutralized quartz sand with 20 or 50% of either bentonite (cation exchange capacity 90 meq 100 g^{-1}, particle size 0·01–1 μm) or kaolinite (CEC 8 meq 100 g^{-1}, size 0·1–5 μm). All mixtures were saturated with Ca^{2+}, washed with excess nitrogen-free nutrient, and buffered at pH 7·5 with 0·01 mol m^{-3} phosphate buffer. Data are given in Table 1. Although growth and nitrogenase activity were detectable in pure sand, rates were very low. Highest carbon and nitrogen yields were obtained when the surface area of the substrate was highest (particle size smallest) and cation exchange capacity highest. Thus although all treatments had adequate water and minerals other than nitrogen, there were large differences in yield. These were thought to relate to surface factors, in particular the interface between particles and the surface polymers of the cyanobacteria; these in turn could affect processes such as ion uptake.

Water content also has a major effect on cyanobacterial activity. Some species such as *Nostoc flagelliforme* and *N. commune* from China (Scherer *et al.* 1984) are very desiccation tolerant and after 2 years of drought can rapidly resume respiration (30 min after rewatering), photosynthesis (6–8 hours) and nitrogen fixation (120–150 hours). Such plants clearly fill an ecological niche. Do they have a role in succession? This depends upon how much nitrogen they fix and release in a form which can be used by other organisms and whether there is enough water to support these other organisms. A succession proceeding towards vascular plants, with the possible exception of certain ephermerals, requires more water than is present in true deserts without a deep water-table accessible to phreatophytes.

Salinity represents no real problem for cyanobacteria, many of which are marine. Where there is much sand movement cyanobacteria may be unable to colonize either on their own or in symbiosis with fungi, forming lichens, which may also be very desiccation tolerant. Lichens may be found in deserts, in dune

TABLE 1. Growth (measured as carbon and nitrogen content) of *Anabaena variabilis* on sand–clay mixtures. Carbon and nitrogen content were determined after 12 days, nitrogenase activity (acetylene reduction) after 10 days. The carbon and nitrogen contents of 100% sand were at the limits of the assay and are thus given in parentheses. After Skujins *et al.* (1987)

Substrate			mg g^{-1} substrate		C_2H_4/nmol $hour^{-1}$ 10 cm^{-2} surface
S	B	K	C	N	
100	0	0	(0·18)	(0·07)	110
80	20	0	1·01	0·26	530
50	50	0	2·68	0·65	680
80	0	20	0·53	0·13	260
50	0	50	0·87	0·21	210

S = sand; B = bentonite; K = kaolinite.

slacks and on coastal rocks. Although various micro-organisms have good particle-binding properties, fixation of sand-dunes tends to be linked with angiosperms such as marram grass; nitrogen-fixing micro-organisms may be associated with roots of such plants, but true symbiotic nitrogen-fixing organisms such as legumes tend to be absent. Grubb (1986) suggests that this may be because they cannot produce 'an exceedingly light and outstandingly dispersible seed' which also contains sufficient 'starter nitrogen'. He notes occasional exceptions including *Lupinus densiflorus* on the coastal range of California. The particular properties of the genus *Lupinus* in this general context are now considered.

One of the success stories in the use of *Lupinus* has been with *L. arboreus* on coastal sand-dunes in the North Island of New Zealand. Here the sand is about 0·008% nitrogen-deficient by any standards. Other nutrients are generally not deficient (see Gadgil 1983, and references therein). Although foredune stabilization is generally achieved using marram grass (given some nitrogen fertilizer) prior to sowing lupins, the latter can successfully bind dunes. Lupins establish readily from seed, nodulate copiously and fix enough nitrogen to support growth of *Pinus radiata* which is planted after the lupins have been killed. Sufficient rhizobia are present in the sand, inoculation being without effect — perhaps it should be noted that *Lupinus* nodulates with a wide variety of rhizobia. This situation would appear to vindicate Grubb's suggestion. The only apparent restriction on successful colonization by lupins in this system is the presence of seed. Is there anything special about lupins? They generally produce long tap roots, favouring both anchorage and mineral uptake from deep layers. They do not normally form mycorrhizal associations (Tester *et al.* 1987); this would be considered by many to be a disadvantage on nutrient-poor soils. However, they readily form proteoid roots which may be an alternative way of obtaining nutrients (see review by Lamont 1982). Proteoid roots appear to be formed mainly by plants (especially the Proteaceae), which have a relatively low demand for nutrients. They have the advantage of not having to supply an endophyte with carbon and being produced locally on parts of root systems where nutrients are available (e.g. litter fragments). They would thus appear to use the host plant's carbon resources effectively, although a cost–benefit analysis of the type carried out for nodulated or mycorrhizal plants does not appear to have been made.

The role of root-associated nitrogen-fixing micro-organisms in the colonization of sand-dunes has been considered many times, but really critical data are rather scant. Non-photosynthetic species require a carbon source — usually a plant. In his biological flora of *Ammophila arenaria* Huiskes (1979) gives references to claims for nitrogen fixation in association with its roots. Some of these should be regarded with caution, e.g. those involving *Azotobacter*, which is not salt tolerant. The case for the saltmarsh plant *Spartina alterniflora* is rather better made (Whiting *et al.* 1986), the authors pointing out the tight coupling between host carbon dioxide fixation and nitrogenase activity. In such systems it appears that the nitrogen-fixing organisms use soluble carbon compounds synthesized recently by the host. Is it also possible that significant nitrogen fixation could be associated

with dead plant material? Recent interest in the proper management of organic waste such as straw has produced evidence that cellulolytic organisms (fungi or bacteria) can live in mixed culture with nitrogen-fixing organisms (Veal & Lynch 1984). Whether such associations occur naturally in pioneer habitats is not known. A further possibility has been raised by the recent evidence of cellulolytic anaerobic nitrogen-fixing bacteria in fresh water and forest soils (Leschine *et al.* 1988). In view of the comparatively small amounts of nitrogen required in early successional stages (see later) any of these organisms could have a significant role in succession.

A rather different system has been reported for inland sand-dunes and sand plains in northern Canada (Nelson *et al.* 1986). Here *Hudsonia tomentosa* (Cistaceae) is a major colonizing species. It is found in sands with poor water retention but relatively good light penetration. It is semi-evergreen and buried leaves and fruits may aid water retention. Associated with this plant (within 1 cm of plant parts), but not with other vascular plants in the same area, were several nitrogen-fixing cyanobacteria including *Nostoc* spp. Unlike the cyanobacteria in desert areas, which form crusts, those associated with *Hudsonia* were found in discrete grains or aggregates of grains sometimes forming green bands down to 22 cm. Whether these would have sufficient light to photosynthesize is very doubtful. Nitrogen-fixation rates were thought to be more water limited than light limited in the field. Although their nitrogen-fixing activity was low, they were considered to be significant in the autecology of *Hudsonia*. Why these cyanobacteria do not colonize areas near other vascular plants in this region is not clear.

Casuarina spp., especially *C. equisetifolia* can have a significant role to play in colonizing base-rich sand-dunes. Like *Lupinus, Casuarina* can form proteoid roots; it may also be mycorrhizal. Discussion is deferred to the next section.

VOLCANIC LAVA

There are relatively well-documented accounts of colonization of sites following volcanic eruption, the most comprehensive being for Krakatoa. The results have been reviewed by Whittaker *et al.* (1989). There are a number of elements in common between the early colonization of Krakatoa and that of Surtsey Island (off Iceland) and La Soufrière in Guadeloupe (Fritz-Sheridan 1987). The first colonizers on all three were mainly (or exclusively) cyanobacteria, including nitrogen-fixing forms, probably brought in by wind. The next stages, however, varied both within and between sites. We shall compare the tropical sites of Guadeloupe and Krakatoa. La Soufrière is a mountain 1456 m high and an eruption in 1976–77 sterilized the south-east flank of the dome. Near the summit, which is shrouded in cloud for most of the year, the soil surface temperature is generally in the range 15–18°C. On the rare clear days it rises to 26–42°C. Whereas on the other volcanoes studied several different species of cyanobacteria were found, the La Soufrière site was mainly colonized by the heterocystous genus *Scytonema*. This nitrogen-fixing organism is very gelatinous with good water-holding properties, although it dries out to a thin papery sheet on clear

days. Its gelatinous nature when hydrated also enables it to resist rain scouring. *Scytonema* not only fixes nitrogen, but also provides a substratum for propagules of the next colonizer, the nitrogen-fixing lichen *Stereocaulon virgatum*. This sequence of two nitrogen-fixing forms is unusual. The lichen develops to a height of 10 cm, reflects heat and enables *Scytonema* to grow at the base of its canopy. The major reason for the success of this system appears to be the persistent cloud cover, maintaining hydration.

On Krakatoa, colonization patterns varied between coastal and inland parts. Inland the successions appear to have begun with gelatinous mats of several cyanobacteria, including nitrogen-fixing forms. These were quickly followed at all levels (the highest point being 735 m) by ferns, rather than lichens. Subsequent development towards forest or scrub varied with site, altitude and occurrence of further eruptions (Whittaker *et al.* 1989). At one site, where the inland forest was dominated by *Timonius compressicaulis*, the thorny creeper *Elaeagnus latifolia* was common on the ground and in the canopy. *Elaeagnus* is an actinorhizal genus (like *Casuarina*) and it would be interesting to know if this species was fixing nitrogen and why the site (presumably) had areas with lower levels of combined nitrogen than others. At coastal sites there is a type of vegetation known as pes-caprae, consisting of low-growing herbs and creepers, including *Ipomoea pes-caprae*. However, also included are some legume species, such as *Canavalia maritima* and *Vigna marina*. Coastal erosion apparently restricts the expanse of pes-caprae. In areas where accretion occurs, *Casuarina equisetifolia* may take on a successional role and persist to form mature woodland.

The excellent review by Whittaker *et al.* (1989) for Krakatoa enables us to examine the suggestion by Grubb (1986) that a limitation on legumes as colonizers may be because their necessarily nitrogen-rich seeds are not light enough for rapid dispersal. Fig. 2 shows how the various species of plant (including cyanobacteria) recorded on all islands, except Anak Krakatoa, are dispersed. Note the preponderance of wind and sea dispersal in the early stages, with birds and bats becoming more important as the populations of these animals build up. The authors suggest that cyanobacteria and ferns arrived by wind and that when they had modified the nature of the soil and microclimate sufficiently, wind-dispersed angiosperms became incorporated. Sea-dispersed plants also arrived early on. Of the 103 species in this category recorded between 1883 and 1893, one-third are legumes. Of the 34 legumes in the species list, 30 are known to be potentially nodulated, one cannot (*Cassia siamea*), two are doubtfully nodulated and for one there is no information (Faria *et al.* 1989). The reason that many of these do not establish well, at least in the early stages, is thought to be the eroding state of the shore. Thus it appears that legume colonization is possible if a seed source sufficiently close for sea dispersal is available. The volcanic islands of Krakatoa are within 40 km of the main Indonesian islands, whereas it is over 2000 km from the east coast of Australia to the sands in New Zealand described above. The natural arrival of *Casuarina equisetifolia*, which has winged seeds, is by wind. It is native to Indonesia. Its role in stabilizing coastal areas in Krakatoa is similar to

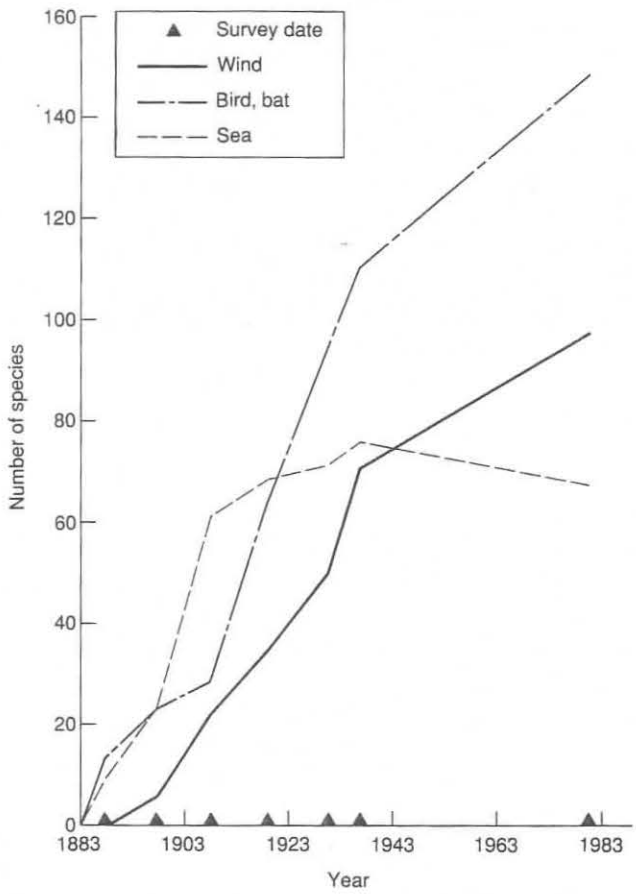

FIG. 2. How colonizing plant species arrived at Krakatoa following volcanic eruption. From Whittaker *et al.* (1989).

that in other areas where it is native or has been exploited for the improvement of arid and saline areas in various parts of the tropics and subtropics (National Research Council 1984).

In the Hawaiian Islands there is a considerable legume flora. However, these native species do not appear to be able to colonize the young volcanic substrates on the island of Hawaii. This could be because of seed dispersal problems, although this possibility has not been explored. These volcanic sites were studied by Vitousek *et al.* (1987) who found that of all the major and minor plant nutrients only addition of nitrogen led to increased growth of the native tree *Metrosideros polymorpha*. The site clearly has major nitrogen-deficiency problems, as do most sites of recent volcanic activity. In this case, however, there is considerable invasion by *Myrica faya*, an exotic plant originally brought to the Hawaiian Islands by Portuguese immigrants. In the absence of *Myrica*, most

nitrogen input is from rainfall ($5\,kg\,N\,ha^{-1}\,year^{-1}$). In similar areas with *Myrica* an additional input of $18\,kg\,N\,ha^{-1}\,year^{-1}$ was estimated by Vitousek *et al.* (1987). Further, the *Myrica* sites contained a considerable amount of biologically available soil nitrogen, whereas non-*Myrica* sites had little. *Myrica* potentially leads to substantial modification of the succession; the whole system is reviewed in Vitousek & Walker (1989). The seed of *Myrica* is dispersed by a variety of birds including exotic species. Although the plants were heavily nodulated, the source of the *Frankia* inoculum is unknown. It would be tempting to suggest, from this *Myrica* study and the work on tree lupins described earlier, that the arrival of the host rather than the endophyte is the limiting factor in establishing legume and non-legume nodulated plants in pioneer situations which are nitrogen-deficient. However, this may not always be the case, since for example, European soils lack the rhizobia necessary to nodulate soybeans (*Glycine max*).

ROCKY AREAS

These occur on coasts, mountains (including volcanoes) and elsewhere. Nitrogen-fixing cyanobacteria occur on, in and under rocks, as long as the substrate is low in nitrogen, sufficient in other minerals, wet at least some of the time and usually not acid. Table 2 summarizes some of the habitats and their limitations. For further details see Hoffmann (1989) and references therein. The cyanobacteria frequently are associated with microalgae and often with lichens. Depending on the environment they can trap mineral and other particles which can lead to the formation of pockets of 'soil' which can be further colonized. How much nitrogen is fixed and what proportion enters the soil pockets with precipitation and dry deposition are largely a matter of conjecture at this stage.

GENERAL CONSIDERATIONS

Almost all primary successions have nitrogen-fixing cyanobacteria among their first colonizers. Fixing both carbon dioxide and nitrogen is a clear advantage if nitrogen is the major limiting nutrient. The need for light may be a disadvantage and it should not be assumed that because visibly green cyanobacterial cells are present they are actively photosynthesizing (symbiotic forms may be dark green

TABLE 2. Rock habitats colonized by cyanobacteria

Type of habitat	Description	Comments on cyanobacteria
Epilith	Exposed surface	Most tolerate temperature extremes and desiccation
Hypolith	Under translucent pebbles down to about 4 cm	May be active down to 0·005% of incident light
Endolith	Colonize or make cavities	Sites very variable in irradiance. Some species may bore into limestone

inside coralloid roots of cycads 1 m below ground). Stal (1985) and Herbert *et al.* (1988) found a rapid attenuation of light penetrating sand, reaching zero at 8–10 mm depth. The total amount of nitrogen fixed by such organisms is likely to be small and may or may not be made available to later successional stages; it may, for example, be leached out following a drying–wetting cycle or lost by denitrification. Thus, the presence of cyanobacteria is not guaranteed to lead to a build-up of combined nitrogen in an ecosystem. Studies by Stal (1985) and others agree that an upper limit of 15 kg N ha^{-1} $year^{-1}$ may be fixed by cyanobacteria. They have a low carbon : nitrogen ratio (about 5–15) and are therefore likely to be mineralized rapidly following death (probably more rapidly than symbiotically fixing structures such as nodules). If we assume a carbon : nitrogen ratio of 5 and a carbon content of 45% of dry weight, then this level of nitrogen fixation would yield 16·6 g dry weight cyanobacterial cells m^{-2}. For colonizing similar to marram grass, the carbon : nitrogen ratio is likely to be closer to 45 : 1 (i.e. overall plant nitrogen concentration is about 1%). Thus, if cyanobacterial nitrogen were all made available for later plant colonizers, it would support about 150 g dry matter m^{-2}. If supplemented by nitrogen from other sources (rain, sea-spray) cyanobacterial fixation could suffice for subsequent angiosperm colonization to a level which is frequently observed.

It appears that much pioneering vegetation remains nitrogen-limited and yet nodulated legumes and actinorrhizal plants are often not among primary colonizers. Evidence cited above for *Lupinus arboreus* and *Myrica faya* supports Grubb's (1986) suggestion that this may be because of lack of seed. However, it seems somewhat surprising that such an obvious niche for nitrogen fixation has not been more widely exploited. We shall end with some speculation as to the consequences such exploitation could produce. In both the examples cited here, as well as in those where nitrogen-fixing plants have been used to hasten recolonization of mine spoils and other man-made wastes (e.g. Dancer *et al.* 1977a, b), accumulation of nitrogen in the ecosystem has been greatly accelerated. Is this necessarily a good thing?

If the supply of combined nitrogen is the rate-limiting step can other resources (phosphorus, potassium, trace elements) be better husbanded? Removal of nitrogen limitation could lead to more rapid cycling of all nutrients with possible losses to the ecosystem in wetter areas. The occasional occurrence (e.g. of *Casuarina equisetifolia*) of nitrogen-fixing plants in primary succession is usually associated with some type of environmental stress such as drought. Amounts of nitrogen fixed may then be limited by water supply. Where nitrogen-fixing plants now occur naturally they are commonly either a small component of a mixture of species or, if dominant or co-dominant, in arid or semi-arid areas, or as part of regeneration after fire. In all cases the total amount of nitrogen fixed per hectare is low (considerably less than 100 kg ha^{-1} $year^{-1}$). For example, Sheehy (1989) calculated that the net nitrogen input from white clover to a grazed pasture in England needs to be about 41 kg ha^{-1} $year^{-1}$ and this could be met by clover occupying about 10% of the surface area of the pasture. Similarly, in stands of

Acacia spp. in Africa and Australia the total nitrogen fixed (in as much as this can be measured!) is likely to be well below 50 kg ha^{-1} $year^{-1}$ (Sutherland & Sprent 1993). High rates of fixation (500 kg ha^{-1} $year^{-1}$) may occur in pure stands of lucerne or grain legumes where most of the nitrogen is removed with the crop. This, ecologically, is a wholly artificial situation.

High rates of fixation in natural systems — such as those following the aggressive colonization by *Leucaena leucocephala* in parts of Hawaii (L. Handley-Raven, unpublished data) could result in excess mineralization and enhanced nitrate is groundwater. In a balanced system nitrogen lost from a legume should be taken up by other plants. This may require a more varied flora than normal in primary colonization. Unfortunately, although there is recent evidence of nitrogen loss from growing (as opposed to damaged or senescent) legumes (Wacquant *et al.* 1989), its quantification, like that of nitrogen fixation in the field, is virtually non-existent. Until we have realistic figures for such processes, the potential of nitrogen-fixing legumes and actinorhizal species in succession remains largely a matter of speculation. Good land management requires that these problems are properly addressed.

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